Amendments to the Specification

Following page 98 and before the claims, please add the paper copy of the Sequence Listing, attached hereto.

In addition, please amend the specification under the provisions of 37 C.F.R §1.121, to comply with the requirements of 37 C.F.R §1.821(d), as follows:

On page 15, please replace the paragraph beginning "*Figure 15* ..." at line 16, and ending with "...respectively;" at line 18, with the following amended paragraph:

Figure 15 shows the molecular dynamic simulation based model of the hexapeptide TFTSDY (SEQ ID NO:1), bound to the secondary binding site of DP IV. Important amino acid residues from the enzyme are light gray, those from the hexapeptide are marked in dark gray, respectively;

On page 15, please replace the paragraph beginning "*Figure 16* ..." at line 19, and ending with "...test;" at line 21, with the following amended paragraph:

Figure 16 shows the prolongation of the half-lifes of GIP, Glucagon, PACAP-27 and PACAP-38 by the hexapeptide TFTSDY (SEQ ID NO:1) in a DP IV (porcine and recombinant human) catalyzed peptide truncation test;

On page 15, please replace the paragraph beginning "*Figure 17* ..." at line 23, and ending with "...line);" at line 24, with the following amended paragraph:

Figure 17 shows the DP IV-catalyzed hydrolysis of RANTES1-15 with (black solid triangle or broken line) or without TFTSDY (SEQ ID NO:1) (black solid square or straight line);

On page 15, please replace the paragraph beginning "*Figure 18* ..." at line 26, and ending with "...square);" at line 27, with the following amended paragraph:

Figure 18 shows the DP IV-catalyzed hydrolysis of GIP with (black solid triangle) or without TFTSDY (SEQ ID NO:1) (black solid square);

On page 15, please replace the paragraph beginning "*Figure 19* ..." at line 29, and ending with "...triangle);" at line 30, with the following amended paragraph:

Figure 19 shows the DP IV-catalyzed hydrolysis of glucagon with (black solid circle) or without TFTSDY (SEQ ID NO:1) (black solid triangle);

On page 21, please replace the paragraph beginning "The amino acid ..." at line 1, and ending with "...YAEGTF" at line 3, with the following amended paragraph:

The amino acid sequences of natural GIP_{1-30} and GIP_{1-6} are:

GIP_{1-30}: YAEGTFISDYSIAMAKIHQQAFVNWLLAQK (SEQ ID NO:2)

GIP_{1-6}: YAEGTF (SEQ ID NO:3)

On page 21, please replace the paragraph beginning "To identify ..." at line 5, and ending with "...(K_i = 0.71mM)." at line 9, with the following amended paragraph:

To identify the secondary binding site, a hexapeptide derived from a consensus sequence of the amino acid sequences of GRF-family peptides was synthesized and its influence on the substrate specificity of DP IV was measured. The selected consensus sequence corresponds to $glucagon_{5-10}$, comprising the amino acid sequence TFTSDY (SEQ ID NO:1). As expected this peptide had only weak influence on the GP-4-Nitroanilide hydrolysis (K_i = 0.71mM).

On page 21, please replace the paragraph beginning "In support ..." at line 10, and ending with "...glucagon." at line 17, with the following amended paragraph:

In support of the results achieved with the GRF family peptides, the truncation half-lifes of GIP, GLP-1, NPY, glucagon or PACAP by DP IV were also changed after preincubation with 160μM TFTSDY (SEQ ID NO:1) (Table 3). No differences could be detected between $Rantes_{1-15}$ and DP IV with or without the

hexapeptide TFTSDY (SEQ ID NO:1) (Table 3). The latter finding shows that the peptide $Rantes_{1-15}$ is too short to reach the secondary binding site and therefore TFTSDY (SEQ ID NO:1) has no effect on its hydrolysis rate. The half-lives of GIP and glucagon in presence of DP IV were prolonged by TFTSDY (SEQ ID NO:1), the strongest influence had TFTSDY (SEQ ID NO:1) on the DP IV-catalyzed truncation of glucagon.

On page 21, please replace the paragraph beginning "Further, ..." at line 18, and ending with "...DP IV." at line 20, with the following amended paragraph:

Further, a modified variant of the hexapeptide TFTSDY (SEQ ID NO:1), TFTDDY (SEQ ID NO:4) was synthesized, studied for docking in the DP IV 3D structural model and tested for its regulatory efficacy to modulate substrate specificity of DP IV.

On page 21, please replace the paragraph beginning "The amino acid ..." at line 21, and ending with "...YAEGTF" at line 22, with the following amended paragraph:

Table 1: Inhibitory effect of TFTSDY (SEQ ID NO:1) on DP IV-catalyzed peptide truncation expressed in K_i-values

On page 22, please replace the paragraph beginning "The hexapeptides ..." at line 3, and ending with "...heptapeptide." at line 14, with the following amended paragraph:

The hexapeptides TFTSDY (SEQ ID NO:1) and TFTDDY (SEQ ID NO:4) were found to be instable in biological fluids, e.g. humen serum or human plasma and/or they were rapidly degraded by proteolytic enzymes in the serum or plasma. Therefore, and in another embodiment of the present invention, a heptapeptide of the sequence

H-Ser-D-Glu-Thr-Gly-D-Val-D-Lys-D-Val-OH

was synthesized. This heptapeptide is not enzymatically degraded in human serum or plasma and is stable in these fluids. A stabilization of the heptapeptide was especially

achieved by the incorporation of D-amino acids in the molecule. It was further shown to be very effective in improving the substrate specificity of DP IV compared to control experiments without the heptapeptide.

On page 24-25, please replace Table 5, beginning at line 16 on page 24, and ending at line 4, page 25 with the following amended table:

Table 5: Kinetic characterization of DP IV-catalyzed substrate hydrolysis by mutants of DP IV in the secondary binding site

Mutation	Test compound	K_m [M]	K_i [M]	kcat [s^{-1}]	k_{cat}/K_m [$M^{-1}*s^{-1}$]
mu 15 DP IV	Gly-Ser-AMC	Not hydrolyzed			
mu 15 DP IV	Gly-Pro-AMC	4.66E-05		1.00E+06	2.15E+10
mu 15 DP IV	V^2GIP(1-4)*		no inhibition		
mu 15 DP IV	S^2GIP(1-6)*		no inhibition		
mu 15 DP IV	Glucagon (1-14)*		no inhibition		
mu 15 DP IV	Leu-Thia-Fum*		6.81E-08		
mu 15 DP IV	TFTSDY* (SEQ ID NO:1)		no inhibition		
mu 15 DP IV	PACAP(1-38)*		3.67E-05		
mu 15 DP IV	Transp 01*		7.69E-08		
mu 15 DP IV	YAESTF amide* (SEQ ID NO:5)		1.14E-06		
mu 16 DP IV	Gly-Ser-AMC	Not hydrolyzed			
mu 16 DP IV	Gly-Pro-AMC	5.02E-05		1.44E+06	2.86E+10
mu 16 DP IV	V^2GIP(1-4)*		no inhibition		
mu 16 DP IV	S^2GIP(1-6)*		no inhibition		
mu 16 DP IV	Glucagon (1-14)*		no inhibition		
mu 16 DP IV	PACAP(1-38)*		3.21E-05		
mu 16 DP IV	Transp 01*		8.55E-08		
mu 16 DP IV	YAESTF amide* (SEQ ID NO:5)		1.06E-06		
mu 16 DP IV	TFTSDY* (SEQ ID NO:1)		no inhibition		
mu 16 DP IV	Leu-Thia Fum*		6.57E-08		
rh wt DP IV	Gly-Ser-AMC	4.4E-04			
rh wt DP IV	Gly-Pro-AMC	3.53E-05		1.66E+06	4.7E+10
rh wt DP IV	V^2GIP(1-4)*		no inhibition		
rh wt DP IV	S^2GIP(1-6)*		no inhibition		
rh wt DP IV	Glucagon (1-14)*		no inhibition		

rh wt DP IV	PACAP(1-27)*	2.28E-04	1.13E-04		
rh wt DP IV	PACAP(1-38)*		3.83E-05		
rh wt DP IV	Transp 01*		5.08E-08		
rh wt DP IV	YAESTF amide* (SEQ ID NO:5)		3.51E-08		
rh wt DP IV	TFTSDY* (SEQ ID NO:1)		no inhibition		
rh wt DP IV	Leu-Thia Fum*	4.26E-05	6.58E-08		
p wt DP IV	Leu-Thia-Fum*	5.98E-05	7.29E-08		
p wt DP IV	PACAP(1-27)*	1.22E-04	5.43E-05		

* The Ki-values were determined in competition of the test compound to the standard substrate GP-4NA (see examples). No inhibition means that the compound doesn't influence the DP IV-catalyzed hydrolysis of the standard substrate GP-4NA.

On page 25, please replace the paragraph beginning "Definitions ..." at line 6, and ending with "...-Thia." at line 11, with the following amended paragraph:

Definitions in table 5:

mu 15	recombinant human DP IV, mutation R560A
mu 16	recombinant human DP IV, mutation W629A
rh wt	recombinant human DP IV, wild type
p wt	porcine kidney DP IV, wild type
Transp 01	RRLSYSRRRF-E-Thia (SEQ ID NO:6)

On page 25, please replace the paragraph beginning "In the present ..." at line 6, and ending with "...-D-Val-OH." at line 15, with the following amended paragraph:

In the present invention a region was identified in the DP IV-protein, which is responsible for the interaction with a hexapeptide, e.g. TFTSDY (SEQ ID NO:1) or TFTDDY (SEQ ID NO:4), or more suitably, a degadation resistent heptapeptide, e.g. H-Ser-D-Glu-Thr-Gly-D-Val-D-Lys-D-Val-OH.

On page 80-81, please replace the paragraph beginning "**Example 1:** ..." at page 80, line 22, and ending with "...conditions." at page 81, line 10, with the following amended paragraph:

Example 1: Determination of the half-life ($t_{1/2}$)

Matrix-assisted laser-desorption ionization time of flight mass spectrometry (MALDI-TOF MS) experiments were carried out at 30°C at pH 7.6 in 0.1 M Tris/HCl (Sigma-Aldrich, Deisenhofen, Germany) buffer with 25μM peptide solution. The degradation fate of peptides was measured by monitoring the signal intensity of the pseudomolecular ion peaks of parent peptides and N-terminal shorted peptides versus time when incubated with 40 mU procine DP IV, recombinant human DP IV or serum DP IV activity. The enzyme was preincubated with hexapeptide TFTSDY (SEQ ID NO:1) or TFTDDY (SEQ ID NO:4) or the heptapeptide H-Ser-D-Glu-Thr-Gly-D-Val-D-Lys-D-Val-OH (15 min, 30°C, 0,016M, 1:1 with DP IV, the concentration of the hexapeptide or the heptapeptide in the reaction mixture was 160 μM). As control served the preincubation of DP IV with 0.01M Tris-buffer (Sigma-Aldrich, Deisenhofen, Germany). The mass spectrometer employed was a Hewlett-Packard G2025 model with a linear time of flight analyzer; samples (4 μL) were mixed 1:1 v/v with matrix (44 mg diammonium-hydrogen-citrate and 30 mg 2',6'-dihydroxyacetophenone in 1 ml aqueous solution containing 50% acetonitrile and 0.05% trifluoroacetic acid; Sigma-Aldrich), transferred to a probe tip and immediately evaporated using the Hewlett-Packard G2024A (Hewlett-Packard, Waldbronn, Germany) sample preparation vacuum chamber. 250 single laser-shot spectra were accumulated. This method of monitoring biodegradation has been validated and allows the general comparison of half-degradation times ($t_{1/2}$) under various conditions.

On page 82, please replace the paragraph beginning "**Example 3:** ..." at line 20, and ending with "...calculated." at line 28, with the following amended paragraph:

Example 3: MALDI-TOF approach

In order to investigate directly the influence of the test compounds TFTSDY (SEQ ID NO:1), TFTDDY (SEQ ID NO:4) and H-Ser-D-Glu-Thr-Gly-D-Val-D-Lys-D-Val-OH on the DP IV-catalyzed peptide hydrolysis the MALDI-TOF assay was used.

As described before (determination of $t_{1/2}$) DP IV and the test compounds were preincubated and the reaction was started by adding the enzyme/hexapeptide mixture

to substrate/buffer mix. The control reaction mixture consisted of buffer, enzyme and substrate. From the curves of the first order exponential the initial rate (v_i) for the control and the reversible inhibited reaction was calculated.

On page 84, please replace the paragraph beginning "*Plasmid construction* ..." at line 11, and ending with "...before." at line 20, with the following amended paragraph:

Plasmid construction and DNA sequencing.
The DP IV encoding region (Δ1-36) plus his6-tag contained in a pcDNA-3.1 vector was amplified using primers DP IV-21 (TCATCGATGCATCATCATCATCATCAT) (SEQ ID NO:7) and DP IV-22 (TAGGTACCGCTAAGGTAAAGAGAAAC) (SEQ ID NO:8) while implementing the restriction sites for KpnI and BspD1. This fragment was digested with the restriction enzymes KpnI and BspD1 as well as the vector pPCR-ScriptCam (Stratagene, USA), afterwards vector and PCR product were ligated and transformed into the E. coli-strain XL-10. Insertion and orientation was confirmed applying restriction enzyme analysis and partial sequencing. That was followed by excision of the DP IV encoding region from the pPCR-ScriptCam vector with the same restriction enzymes KpnI and BspD1 and its ligation into the Pichia vector pPICaC, which was also treated with the same restriction enzymes before.

On page 84-85, please replace the paragraph beginning "In the present ..." at page 84, line 22, and ending with
"...GGTTACGTACCCTCGCTATGACCAGCCCCAAATTGC." at page 85, line 2, with the following amended paragraph:

Site directed mutagenesis:
Single amino acid mutations were carried out with the Quick Change Site-directed Mutagenesis Kit from Stratagene (USA). Following primers were used to introduce the mutations:
R310A-DP IV:
DP IV-84 GACATGGGCAACACAAGAAGCAATTTCTTTGCAGTGGC (SEQ ID NO:9)

DP IV-85 GCCACTGCAAAGAAATTGCTTCTTGTGTTGCCCATGTC (SEQ ID NO:10)
R560A-DP IV:
DP IV-73: GCAGACACTGTCTTCGCACTGAACTGGGCCACTTACC (SEQ ID NO:11)
DP IV-74b: GGTAAGTGGCCCAGTTCAGTGCGAAGACAGTGTCTGC (SEQ ID NO:12)
W629A-DP IV:
DP IV-75: GCAATTTGGGGCTGGTCATAGCGAGGGTACGTAACC (SEQ ID NO:13)
DP IV 76: GGTTACGTACCCTCGCTATGACCAGCCCCAAATTGC (SEQ ID NO:14).

On page 91, please replace the paragraph beginning "**Example 9:** ..." at line 24, and ending with "...site." at line 30, with the following amended paragraph:

Example 9: Docking of GIP; VIP and glucagons to DP IV

Several oligopeptides such as GIP, VIP, glucagon and others are hydrolysed by DP IV and therefore it is clear, that these substrates are docking to DP IV and reaching the active site. Extensive docking investigations by means of molecular dynamics simulations were done using the old model. From these studies the amino acid sequences of the hexapeptides, TFTSDY (SEQ ID NO:1) and TFTDDY (SEQ ID NO:4) and the degradation stabilized heptapeptide H-Ser-D-Glu-Thr-Gly-D-Val-D-Lys-D-Val-OH were derived and its ability to protect oligopeptide substrates from their interaction with a secondary binding site.

On page 92, please replace the paragraph beginning "The binding ..." at line 1, and ending with "...site." at line 5, with the following amended paragraph:

Results

The binding and hydrolysis of small dipeptide substrates were only slightly influenced when DP IV was preincubated with the hexapeptides TFTSDY (SEQ ID NO:1) or TFTDDY (SEQ ID NO:4) or the degradation stabilized heptapeptide H-Ser-D-Glu-

Thr-Gly-D-Val-D-Lys-D-Val-OH but the affinity of larger oligopeptides such as GIP, VIP, glucagon and others was considerably reduced. These experiments clearly prove the existence of a secondary binding site.

On page 94, please replace the paragraph beginning "Based ..." at line 8, and ending with "...Tables 7 to 9." at line 10, with the following amended paragraph:

Based on these results analogous docking studies were performed with VIP, glucagon and the hexapeptide TFTSDY (SEQ ID NO:1) (Figs. 12 to 15) The results are summarized by listing the most attractive interactions in Tables 7 to 9.

On page 94, please replace the paragraph beginning "Moreover, ..." at line 17, and ending with "...site." at line 21, with the following amended paragraph:

Moreover, the results of these studies confirm the proposed docking of Lys-Z-nitro-Pyrrolidine, e.g. the interaction of the nitro-group with AR560. Wher the oligopeptide ligands have an Asp in third or fourth position in their amino acid sequence, a salt bridge with R560 is formed. By docking arrangement of the hexapetide TFTSDY (SEQ ID NO:1) (Fig. 15), it was proven that this hexapeptide indeed prevents binding of oligopeptide ligands to the active site.